REMARKS

Claims 1, 4, 6-11 and 13-17, and 40-51 are pending in the application. Claims 12 and 18-39 are withdrawn as a result of the restriction requirement. Claims 40-51 are new with this response. Claims 2, 3 and 5 have been cancelled

Claim 1 has been amended to incorporate the elements of claim 2 and to clarify that the cleavage cite is cleavable by a human Granzyme B. Support for this amendment can be found throughout the specification.

New claims 40-51 are supported by, for example, cancelled claim 3 and claims 4-17.

Support for the amendment to claim 4 is found throughout the specification and specifically, for example, the original claim and page 9.

Applicants thank the Examiner for the reconsideration and withdrawal of the previously pending rejections under 35 U.S.C. § 103 and the various objections to the specifications and claims.

Objections to the Specification

B. Objection to Disclosure for Alleged Conflicting Sequence Structure

The Examiner has maintained the objection to the specification for the disclosure of alleged conflicting information regarding the sequence of GrB-H6 and the structure of pro-IEGR-GrB-H6 (SEQ ID NO: 1). Applicants respectfully disagree with the objection and provide the following further clarification.

The Examiner states that SEQ ID NO:1 does not contain an Ile 21 residue or a Tyr247 residue as described on page 32 of the specification for pro-IEGR-GrB-H6. Amino acid 8 for SEQ ID NO. 1 corresponds to Ile21 in Granzyme B (E.C. 3.4.21.79) Attached hereto as Exhibit A is the sequence of human and mouse Granzyme B as provided for Enzyme Commission No. EC 3.4.21.79, Release 34, February 2004 (*see*

Specification, ¶ 26). As shown in Exhibit A, Granzyme B has Ile at position 21 and Tyr 247 as correctly identified in SEQ ID NO:1. In their previous amendment, Applicants amended page 32 of the specification to show correct the position of Tyr 246 to Tyr 247.

SEQ ID NO:1 correctly shows the construct pro-IEGR-GrB-H6. The sequence begins with the seven amino acids of the Fxa recognition sequence as described. Amino acids 8-234 correspond to amino acids Ile 21 through Tyr 247 of Granzyme B. The three amino acids prior to the His tag are a part of the disclosed T7 cloning vector in which the His tag was cloned. Specifically, they correspond to restriction sites, including EcoR1 site. (*see* page 33, second paragraph).

In the Office Action, the Examiner comments the Applicants' previous argument fails to clarify the sequence of the constructs named GrB-H6 or GrB. Applicants submit that Example 1 sufficiently describes these constructs. Here, the specification teaches that the various inactive pro-Granzyme B constructs were prepared using the sequence from Ile21 to Tyr 246 of activated E.C. 2.4.21.79. As explained above, this sequence is placed after the sequence MGSIEGR and is followed by an H6 tag (including a few amino acids from the cloning vector). Therefore, the sequence of GrB-H6 refers to the sequence following MGSIEGR of SEQ ID NO:1. GrB-H6 is used consistently throughout the specification to refer to the same sequence. As shown in the attached sequence alignment, all of the constructs shown throughout the specification are labeled with the name of the recognition sequence, followed by the GrB-H6 sequence. When a variant of the sequence is used, the nomenclature includes a reference to the specific variant; *e.g,* GrB-H6-C228F. This nomenclature is used consistently throughout the specification.

Accordingly, Applicants respectfully request withdrawal of objections to the alleged conflicting sequence disclosure.

Rejections under 35 USC §112, second paragraph

Claim 5 stands rejected as indefinite for allegedly failing to define the terms P1', P2', and P3'. Claim 5 has been cancelled, which renders the rejection moot.

Rejections under 35 USC §112, first paragraph, enablement

Claims 1-11 and 13-17

Claims 1-11 and 13-17 stand rejected under 35 USC 112, first paragraph, for alleged lack of enablement. According to the Examiner, the specification does not reasonably provide enablement for a method to cleave a fusion protein comprising any Granzyme B cleavage motif using any Granzyme B enzyme having any structure.

Applicants have amended claim 1 to reflect that the Granzyme B cleaves the fusion protein after a narrow genus of cleavage sites that are capably of being cleaved by human Granzyme B. New claim 40, which is based upon former claim 3, is directed to 24 particular motifs, many of which appear in the prior art. *See* Casciola-Rosen, 1999 (Table 1). These amendments address the Examiner's concern that the method is directed to any fusion protein because the claims are now directed to a fusion proteins having a specific subset of cleavage sites. The amendments also addresses the Examiner's concern that the method uses any protein having the protease activity of any Granzyme B. The cleavage site is cleavable by human Granzyme B, although other Granzyme B orthologs may also cleave the cleavage site.

In order to fully address the rejection for lack of enablement, Applicants set forth an analysis of the claim under the eight factors of *In re Wands,* 858 F.2d 731 (Fed. Cir. 1988). On balance, the analysis shows that the present specification enables the full scope of the pending claims.

A. The nature of the invention and the breadth of the claims

The claims are directed to a method of producing a polypeptide, but not to a specific polypeptide per se. The method includes cleavage of a fusion protein at a specific cleavage site that is capable of being cleaved by a well-known enzyme – human Granzyme B. With the amendment to claim 1, and the addition of new claims 40-51, the specific cleavage site has been narrowly defined by a narrow subset of possible motifs.

The Examiner expresses concern that the claims are directed to any protein having any Granzyme B protease activity. Applicants respectfully disagree because the

specification provides sufficient information about the structure and function of human Granzyme B. Moreover, the claims have been amended to reflect that the fusion protein is cleavable by human Granzyme B. A specific Granzyme B sequence is not required when the level of skill in the art is high and there is sufficient public information about the sequence. *See Monsanto v. Scruggs,* 459 F.3d 1328 (Fed. Cir. 2006) (even though only one example of the entire genus of CaMV promoters was described in the specification, no specific gene sequence needed to be claimed for someone of ordinary skill in the art to understand how to make and use the invention; "specific sequences are not required because CaMV is well-known and well-documented. ... The fact that some experimentation may be necessary to produce the invention does not render the '605 patent invalid for lack of enablement.")(emphasis in original).

B. The presence or absence of working examples

The specification contains a number of working examples. The examples include a number of cleavage sites that are cleavable by human Granzyme B:

Example 3: IEAD and IEPD

Example 4: IEPD

Example 5: LEED, VEID, YVAD, IEPD (*see also* FIG. 2)

Example 8: IEPD, IEPD↓SP, IQAD↓SP, IQAD↓SG; VGPD↓FG, VGPD↓SP IEPD↓TQ, IEPD↓IV; IEPD↓EP, IEPD↓EG;

The examples show cleavage of substrates with these cleavage sites by Gr-B and Gr-B-H6 C228F (Examples 4, 5, and 8). Also shown is the cleavage of the self activating enzymes: Pro-IEPD-GrB-H6, Pro-IEGR-GrB-H6; Pro-IEAD-GrB-H6; (Example 3).

C. The amount of direction or guidance provided by the specification

In addition to the examples described above, the specification provides ample guidance on making and using the invention. The examples show construction of vectors for producing GrB-H6 and variants, including the self-cleaving constructs. The specification also teaches the expression and purification of the peptides.

D. The state of the prior art

The prior art is sufficiently advanced such that Granzyme B and several variants of human Granzyme B and several species are well know. For example, attached hereto is a copy of UniProtKB/Swiss-Prot P10144 (GRAB_HUMAN)(Ex. B), which summarizes some of the known information about human Granzyme B prior to the filing of the present application (all cited references dated prior to filing application). The structure and function of Granzyme B is well characterized, including glycosylation sites. disulphide bonds, and secondary structure. Three natural variants are described. Also, Harris, *et al.* teaches variants R1192A and R192E. Casciola-Rosen, J. Exp. Med. (1999), which is of record in the application, teaches twenty cleavage sites that are cleavable by human Granzyme B. In addition, Thornberry, *et al.*, J. Biol. Chem., 272(29):17907-17911-17911 (1997) (copy provided with Supplemental Information Disclosure Statement submitted herewith) shows that methods of identifying specific Granzyme B cleave motifs were known in the art prior to filing the application.

The prior art also included the Granzyme B nucleotide and amino acid sequences for both mouse and rat. Attached as Ex. C are alignments of human Granzyme B with rat and mouse Granzyme B.

E. The relative skill of a person of ordinary skill in the art

The level of skill in the art is high, such as person with a doctorate degree in molecular biology or similar disciple along with post-doctorate or equivalent work experience. The skilled artisan would be proficient with the experimental techniques necessary to perform all of the experiments described in the specification. One of skill in the art would also understand the significance of protein structure, and be able to identify conserved regions in Granzyme B based upon a comparison of sequences from different species.

F. The predictability or unpredictability of the art

While the Patent Office has generally taken the position that biotechnology is unpredictable, Applicants submit that the identification of particular human Granzyme B

cleavage sites and identification of Granzyme B enzymes that cleave these cleavage sites would be routine.

G. The quantity of experimentation necessary

Attached as Ex. C is are alignments of the human with rat and human with mouse Granzyme B. When conservative substitutions are used, the rat and mouse sequences are over 85% identical to human. Because the invention is directed to a method, and not to a peptide itself, the amount of experimentation necessary to practice the full scope of the claims is not undue. *Monsanto v. Scruggs* at 1348.

The examiner recognizes that recombinant and mutagenesis techniques are known. *See* Office Action mailed January 16, 2008, p. 7. Applicants respectfully submit that only routine experimentation would be necessary to screen the limited number of motifs described in the claims for cleavage with human Granzyme B. Simply because experimentation is time consuming or difficult does not render the experimentation undue. *Falko-Gunther Faulkner v. Inglis*, 448 F.3d 1357 (Fed. Cir. 2006). *Ex parte Kubin*, 83 USPQ2d 1410, 1415 (Bd. Pat. App. & Int. 2007).

Accordingly, Applicants respectfully submit that, weighing all the *Wands* factors, on balance, one can readily conclude that the one of skill in the art can make and use the invention without undue experimentation.

Claims 7 and 8

Claims 7 and 8 stand rejected because, according to the Examiner, the specification does not reasonably provide enablement for producing authentic somatotrophin, glucagon, insulin interferon or Granzyme B by cleaving a fusion protein comprising these proteins with Granzyme B. The Examiner has not, however, provided any information or basis for the rejection. For this reason alone, Applicants request that the rejection be withdrawn.

In addition, as discussed above, the Example 3 shows the self-activating cleavage of IEAD-GrB-H6 and IEPD-GrB-H6. Both of these peptides include a Granzyme B cleavage site that is cleavable by human Granzyme B and both peptides include the sequence of GrB-H6 (See Ex. A). Accordingly, the specification provides specific examples of cleavage of a fusion protein comprising Granzyme B with Granzyme B. The specification also provides examples of cleavage of fusion proteins containing Apolipoprotein A1 (pp. 57-68). With regard to the remainder of the proteins of interest, Applicants submit that one of skill in the art could readily make and use the appropriate vectors to produce the fusion proteins comprising the proteins of interest.

With the amendments to the claims and the above discussion, Applicants submit that the claims are sufficiently enabled such that one of skill in the art could practice the invention without undue experimentation.

Accordingly, Applicants request that the rejection of claims 7 and 8 be withdrawn.

Rejections under 35 USC §112, first paragraph, written description

Claims 1-11 and 13-17

Claims 1-11 and 13-17 stand rejected under 25 U.S.C. 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that inventors had possession of the claimed invention at the time of filing.

What is needed to support generic claims to biological subject matter depends on a variety of factors, such as the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, the predictability of the aspect at issue, and other considerations appropriate to the subject matter. *Capon v. Esshar*, 418 F. 3d. 1349, 1359 (Fed. Cir 2004). It is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention. *See id., citing, In re Angstadt*, 537 F.2d 498, 504 (CCPA 1976).

Applicants respectfully submit that the specification adequately discloses the invention such that it is readily apparent that the Applicants had possession of the invention at the time that the application was filed. As addressed above, the claims are directed to a method, and not to any particular sequence or variant. Moreover, the claims have been amended to reflect a specific set of cleavage sites that are capable of being cleaved by human Granzyme B. Applicants respectfully submit that the exact enzyme used for cleaving is not a critical feature of the invention. Therefore, the enzyme does not need to be described, as the Examiner appears to suggest, by a structure, function and a representative number of examples, to the same extent as if the Applicants were claiming the enzyme.

Moreover, the Specification provides sufficient structure and function of the Granzyme B for use in the claimed method. For instance, the function is particularly recited as being capable of cleaving fusion protein and a limited subset of Granzyme B cleavage sites that are capable of being cleaved by Human Granzyme B. Accordingly, Applicants have described their invention such that it is clear that they had possession of the claimed method.

The present claims are similar to those in Example 16, "Process Claim Where Novelty Resides in Process Steps," of the PTO's Written Description Training Materials, Rev. 1, March 28, 2008. In that example, the claim is directed to a method of introducing a variety of nucleic acids into mitochondria of mammalian cells. Practicing the hypothetical method claim of the example requires a defined chemical compound, a

nucleic acid, and mammalian cells. The exemplary specification describes the structure of compound X and one nucleic acid (the ß-galactosidase gene) used to transform mitochondria. A comparison of Example 16 to the present specification reflects that the claims meet the written description requirement.

Example 16 concludes that that the claim meets the written description requirement even though the specification only provides one example of a nucleic acid that can be used to transform mitochondrial cells. The example specification provides only a single example of an actual reduction to practice of a species within the claimed genus; i.e., transformation of mitochondria with DNA encoding ß-galactosidase. In the present situation, the specification provides several examples with GrB-H6, GrB-H6-C228 variants, and a number of self-cleaving Granzyme B constructs.

In Example 16, it is reported that the level of skill and knowledge in the art is such that those skilled in the art know of numerous nucleic acids that could potentially be complexed with compound X (itself a known compound) to be used in the claimed method to transform the mitochondria of numerous mammalian cells. Example 16 explicitly states that although the sequences of these nucleic acids are not disclosed in the specification, a patent application is not required to reproduce knowledge that is available in the art. *See also Monsanto v. Scruggs* at 1348. Likewise, in the present situation, while the specification provides the specific sequence for human Granzyme B and variants, one of skill in the art would be aware of numerous potential Granzyme B orthologs that can be used for cleavage.

In Example 16, it is reported that the degree of predictability within the claimed genus is high because introduction of a nucleic acid into mitochondria is disclosed to depend on complexing with compound X and contacting the complex with mammalian cells under specified conditions. According to the example specification, those conditions would be expected to result in transformation regardless of which nucleic acid is complexed and contacted with cells. In the present situation, the specification sets forth the reaction conditions for cleaving using GrB-H6, the variants and the self-cleaving constructs. One of skill in the art would expect cleavage by human Granzyme B or an

ortholog. The fact that some of the Granzyme B orthologs may not work with every claimed cleavage site does not defeat patentability of the invention. *See Capon v. Esshar* at 1359 ("It is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention.")

Example 16 concludes that those of ordinary skill in the art of mammalian cell transformation would recognize the inventor to have been in possession of the claimed method at the time of filing. Likewise, in the present situation, it can reasonably be concluded that one of skill in the art would recognize that the inventors had possession of the invention at the time of filing the present application.

Accordingly, Applicants respectfully request Examiner to withdraw the rejection of claims 1-11 and 13-17 for lack of written description.

Claims 4 and 5

Claims 4 and 5 stand rejected as lacking written description. Claim 5 has been cancelled, and claim 4 has been amended so that the claim no longer refers to P1'. With the cancellation of claim 5 and the amendment to claim 4, Applicants submit that the rejection has been traversed.

Claims 7 and 8

Claims 7 and 8 stand rejected as lacking written description. The examiner asserts that the specification does not describe methods for cleaving fusion proteins having the recited polypeptides. Applicants respectfully disagree and direct the Examiner to the specification, page 11, middle paragraph, as describing each of the polypeptides recited in the claims as a polypeptide of interest.

Accordingly, Applicants request that the rejection be withdrawn.

Rejections under 35 USC §102

Claims 1-4, 9-11, 16 and 17

Claims 1-4, 9-11, 16 and 17 stand rejected under 35 U.S.C. 102(a) as being anticipated by Harris, *et al.*, 1998. According to the Examiner, Harris, *et al.* teach a method of making a protein of interest, which is a "linker+residues 198-406 of the pIII coat protein of a M13 bacteriophage." (*See* Office Action, p. 11). Applicants respectfully disagree with the rejection.

Harris, *et al.* does not anticipate the present invention because Harris, *et al.* does not make a protein of interest in authentic form as presently claimed. Instead, Harris, *et al.* describes the cleavage of a variety of short synthetic amide substrates produced via a combinatorial library as shown in Tables 2 and 3. Harris, *et al.* teaches the determination of substrate specificity, but not to the production of any particular peptide. The protein product that is produced by the cleavage reaction is Harris, *et al.* is a protein having a linker sequence. The protein is not in authentic form as described in the present specification, which states as follows:

> As used herein, the term "authentic form" refers to a polypeptide which comprises the amino acid sequence thereof without any additional amino acid residues. As described above, a major problem associated with several of the presently applied enzymes for fusion protein cleavage is that spurious or extraneous amino acids frequently remains attached to the cleaved polypeptide product, i.e. resulting in a polypeptide which is not in an "authentic form". Thus, in the present context the polypeptide of interest in authentic form refers to a polypeptide having the same primary amino acid sequence ***as that encoded by the native gene sequence coding for the polypeptide of interest, i.e. it does not contain any non-native amino acids.*** The term "native gene sequence" is not necessarily a gene sequence that occurs in nature, but it may also be partly or completely artificial. Likewise it will be appreciated that a polypeptide of interest in authentic form not necessarily is a polypeptide that occurs in nature, but it may also be partially or completely artificial. In contrast, a "non-authentic" polypeptide contains at least one amino acid which is not encoded for by the native gene sequence coding for the polypeptide of interest.

Specification, pp. 6-7 (emphasis added). Harris, *et al.* does not anticipate the present invention because the method described in Harris, *et al.* does not produce a polypeptide in authentic form as claimed. The peptides after the cleave site in Harris, *et al.* are not an authentic portion of the pIII coat protein of M13 bacteriophage. In particular, the

peptides XXAGPGGG are not encoded a native gene sequence of the M13 bacteriophage. Accordingly, because Harris, *et al.* does not teach each and every element of claim 1, and the claims depending therefrom, Harris, *et al.* does not anticipate claim 1 and all of the dependent claims.

Rejections under 35 USC §103

Claims 1-4, 9-11, 16 and 17

Claims 1-4, 9-11, 16 and 17 stand rejected under 35 U.S.C. 103(a) as obvious over Azad, *et al.* in view of Harris, *et al.* According to the Examiner, it would have been obvious to a person of ordinary skill in the art to modify the fusion protein of Azad, *et al.* to incorporate the motif IEAD, as taught by Harris, *et al.* (FIG. 5D) between the GST fusion partner and nef27, and then generate nef27 by cleaving the fusion protein with Granzyme B. The examiner finds the motivation to combine Azad, *et al.* and Harris, *et al.* from the desire to produce nef27.

Applicants respectfully submit that the combination of Azad, *et al.* and Harris, *et al.* does not render obvious the method of claim 1, and the methods of the remainder of the dependent claims. While applicants agree with the Examiner's conclusion of a general motivation to produce nef27, that motivation alone is not sufficient to render the presently claimed method obvious. The motivation that the Examiner cites is simply the desire to make polypeptides in authentic form, be it nef27 or any other polypeptide. This general motivation does not lead one of ordinary skill in the art to choose the method of *Harris, et al.* for producing polypeptides.

As discussed above, Harris, *et al.* does not teach producing a polypeptide of interest in authentic form. Instead, Figure 5 and the remainder of Harris, *et al.* discloses a six amino acid motif IEAD↓AL (P4 P3 P2 P1 ↓ P1' P2') that is explained as essential for Granzyme B cleavage (see abstract and Figure 5). The amino acids following the cleavage site, including P1', P2' and the remainder of the linker (AGPGGG) are not part of the authentic polypeptide sequence of the pIII coat protein of M13 bacteriophage. Indeed, Harris, *et al.* teach away from the present invention because Harris, *et al.* teach the necessity of P1' and P2' amino acids. Therefore, one of skill in the art would not

look to Harris, *et al.* as teaching a method for the production of the nef27 polypeptide of Azad, *et al.* Therefore, the combination of Harris, *et al.* and Azad, *et al.* do not render obvious claim 1 or any of the claims depending therefrom.

Claims 1-4, 9-11, 16 and 17 stand rejected under 35 U.S.C. 103(a) as obvious over Azad, *et al.* and Harris, *et al.* in view of Boutin, *et al.* According to the Examiner, Boutin, *et al.* teaches a Granzyme B cleavage motif comprising D or E at P4'. Applicants respectfully submit that combination of Azad, *et al.* and Harris, *et al.* does not render obvious claim 1 for the reasons addressed above. Boutin, *et al.* does not add to the case of obviousness against claim 1. Also, the Examiner suggests that Boutin, *et al.* includes a protein beginning with Met-Gly at the N-terminus and that the enzyme calcineurin B has an E at P4'. Applicants disagree because as shown in Table 3, none of the proteins begin with Met, and calcineurin G does not have E at P4'. Indeed, the first line of the Abstract states that "N-myristolyation is an acylation process absolutely specific to the N-terminal amino acid glycine in proteins." Accordingly, Boutin, *et al.* has nothing to do with enzyme cleave reactions as in the present invention.

Claims 1-4, 9-11, 16 and 17 stand rejected under 35 U.S.C. 103(a) as obvious over Azad, *et al.* and Harris, *et al.* in view of Sigma Inc. 1998 or Pharmacia, Inc. Applicants understand that Sigma Inc and Pharmacia Inc are cited as teaching the immobilization of proteases. Sigma Inc. and Pharmacia, Inc. do not, however, cure the deficiency of the combination of Azad, *et al.*, and Harris, *et al.* to render obvious claim 1. Therefore, the combination of Sigma, Inc. or Pharmacia, Inc. with Azad, *et al.* and Harris, *et al.* do not render obvious any of the claims depending from claim 1.

CONCLUSION

Applicants submit that all of the rejections and objections in the Office Action have been addressed with the forgoing arguments and amendments. Applicants do not waive any argument by failing to make the argument here. Applicants expressly reserve the right to reassert the above arguments, or assert additional arguments in the future.

Applicants respectfully contend that all conditions of patentability are met in the pending claims as amended. Allowance of the claims is thereby respectfully solicited.

If the Examiner believes it to be helpful, he is invited to contact the undersigned representative by telephone at 312 913 0001.

Respectfully submitted,

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Exhibit A

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                        10        20        30        40        50        60
                         |         |         |         |         |         |
SeqID_6      ------------MGSIEP-DIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
SeqID_7      ------------MGSIEP-DIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
SeqID_5      ------------MGSIEP-DIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
SeqID_4      ------------MGSIEP-DIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
SeqID_8      ------------MGSIEP-DIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
SeqID_2      ------------MGSIEP-DIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
SeqID_3      ------------MGSIEA-DIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
SeqID_1      ------------MGSIEG-RIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
SeqID_57     --------------------IIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
Humanx9      MQPILLLLLAFLLLPRADAGEIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
                                  ************************************

                        70        80        90       100       110       120
                         |         |         |         |         |         |
SeqID_6      TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
SeqID_7      TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
SeqID_5      TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
SeqID_4      TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
SeqID_8      TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
SeqID_2      TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
SeqID_3      TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
SeqID_1      TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
SeqID_57     TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
Humanx9      TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
             ************************************************************

                       130       140       150       160       170       180
                         |         |         |         |         |         |
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SeqID_7      TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRHY
SeqID_5      TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRHY
SeqID_4      TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRHY
SeqID_8      TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRHY
SeqID_2      TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRHY
SeqID_3      TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRHY
SeqID_1      TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRHY
SeqID_57     TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRHY
Humanx9      TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRHY
             ************************************************************

                       190       200       210       220       230       240
                         |         |         |         |         |         |
SeqID_6      YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRATTKVSSFVHWI
SeqID_7      YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRAVTKVSSFVHWI
SeqID_5      YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRAATKVSSFVHWI
SeqID_4      YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRASTKVSSFVHWI
SeqID_8      YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRAFTKVSSFVHWI
SeqID_2      YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRACTKVSSFVHWI
SeqID_3      YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRACTKVSSFVHWI
SeqID_1      YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRACTKVSSFVHWI
SeqID_57     YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRAFTKVSSFVHWI
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Humanx9       YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRACTKVSSFVHWI
              ************************************************** **********

                      250
                       |
SeqID_6       KKTMKRYLNSHHHHHH
SeqID_7       KKTMKRYLNSHHHHHH
SeqID_5       KKTMKRYLNSHHHHHH
SeqID_4       KKTMKRYLNSHHHHHH
SeqID_8       KKTMKRYLNSHHHHHH
SeqID_2       KKTMKRYLNSHHHHHH
SeqID_3       KKTMKRYLNSHHHHHH
SeqID_1       KKTMKRYLNSHHHHHH
SeqID_57      KKTMKRY---------
Humanx9       KKTMKRY---------
              *******
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Exhibit B

☆ Reviewed, UniProtKB/Swiss-Prot **P10144** (GRAB_HUMAN)
Last modified January 20, 2009. Version 107.

Names and origin

Protein names	*Recommended name:* **Granzyme B** EC=3.4.21.79 *Alternative name(s):* Granzyme-2 T-cell serine protease 1-3E Cytotoxic T-lymphocyte proteinase 2 Short name=Lymphocyte protease SECT Cathepsin G-like 1 Short name=CTSGL1 CTLA-1 Fragmentin-2 Human lymphocyte protein Short name=HLP C11
Gene names	Name: **GZMB** Synonyms: CGL1, CSPB, CTLA1, GRB
Organism	**Homo sapiens (Human)**
Taxonomic identifier	9606 [NCBI]
Taxonomic lineage	Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Mammalia › Eutheria › Euarchontoglires › Primates › Haplorrhini › Catarrhini › Hominidae › Homo

Protein attributes

Sequence length	247 AA.
Sequence status	Complete.
Sequence processing	The displayed sequence is further processed into a mature form.
Protein existence	Evidence at protein level.

General annotation (Comments)

Function	This enzyme is necessary for target cell lysis in cell-mediated immune responses. It cleaves after Asp. Seems to be linked to an activation cascade of caspases (aspartate-specific cysteine proteases) responsible for apoptosis execution. Cleaves caspase-3, -7, -9 and 10 to give rise to active enzymes mediating apoptosis.
Catalytic activity	Preferential cleavage: -Asp-|-Xaa- >> -Asn-|-Xaa- > -Met-|-Xaa-, -

	Ser-|-Xaa-.
Subcellular location	Cytoplasmic granule. Note= Cytoplasmic granules of cytolytic T-lymphocytes and natural killer cells.
Induction	By staphylococcal enterotoxin A (SEA) in peripheral blood leukocytes.
Sequence similarities	Belongs to the peptidase S1 family. Granzyme subfamily. Contains 1 peptidase S1 domain.

Ontologies

Keywords

Biological process	Apoptosis Cytolysis
Coding sequence diversity	Polymorphism
Domain	Signal
Molecular function	Hydrolase Protease Serine protease
PTM	Glycoprotein Zymogen
Technical term	3D-structure Direct protein sequencing

Gene Ontology (GO)

Uncategorized	granzyme B activity Inferred from Experiment. Source: Reactome
Biological process	cleavage of lamin Inferred from direct assay. Source: UniProtKB cytolysis Inferred from electronic annotation. Source: UniProtKB-KW proteolysis Inferred from electronic annotation. Source: InterPro
Cellular component	cytosol Inferred from Experiment. Source: Reactome immunological synapse Traceable author statement. Source: UniProtKB nucleus Traceable author statement. Source: UniProtKB
Molecular function	protein binding Inferred from physical interaction. Source: UniProtKB serine-type endopeptidase activity Traceable author statement. Source: UniProtKB

Complete GO annotation...

Sequence annotation (Features)

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier
Molecule processing					
Signal peptide	1 – 18	18	Ref.13		
Propeptide	19 – 20	2	Activation peptide Ref.10 Ref.11 Ref.12		PRO_0000027399
Chain	21 – 247	227	Granzyme B		PRO_0000027400
Regions					
Domain	21 – 245	225	Peptidase S1		
Sites					
Active site	64	1	Charge relay system		
Active site	108	1	Charge relay system		
Active site	203	1	Charge relay system		
Amino acid modifications					
Glycosylation	71	1	N-linked (GlcNAc...)		
Glycosylation	104	1	N-linked (GlcNAc...) Potential		
Disulfide bond	49 ↔ 65				
Disulfide bond	142 ↔ 209				
Disulfide bond	173 ↔ 188				
Natural variations					
Natural variant	55	1	Q → R: dbSNP rs8192917.		VAR_018371

				Ref.2 Ref.7 Ref.16		
☐	Natural variant	94	1	P → A: dbSNP rs11539752. Ref.2 Ref.6	‖	VAR_047409
☐	Natural variant	247	1	Y → H: dbSNP rs2236338. Ref.16 Ref.8	|	VAR_018381

Experimental info

☐	Sequence conflict	72	1	V → G in AAA52118. Ref.3	‖	
☐	Sequence conflict	212	1	V → C in AAB59528. Ref.4	‖	

Secondary structure

1 247

Helix Strand Turn

Details...

Sequences

	Sequence		Length	Mass (Da)
☐	P10144-1 [UniParc].	FASTA	247	27,688

Last modified July 1, 1989. Version 1.
Checksum: 684FF605D6F2F4FB

```
        10         20         30         40         50         60
MQPILLLLAF LLLPRADAGE IIGGHEAKPH SRPYMAYLMI WDQKSLKRCG GFLIQDDFVL

        70         80         90        100        110        120
TAAHCWGSSI NVTLGAHNIK EQEPTQQFIP VKRPIPHPAY NPKNFSNDIM LLQLERKAKR

       130        140        150        160        170        180
TRAVQPLRLP SNKAQVKPGQ TCSVAGWGQT APLGKHSHTL QEVKMTVQED RKCESDLRHY

       190        200        210        220        230        240
YDSTIELCVG DPEIKKTSFK GDSGGPLVCN KVAQGIVSYG RNNGMPPRAC TKVSSFVHWI

KKTMKRY
```

« Hide

References

« Hide 'large scale' references

[1] **"Induction of mRNA for a serine protease and a beta-thromboglobulin-like protein in mitogen-stimulated human leukocytes."**
Schmid J., Weissmann C.
J. Immunol. 139:250-256(1987) [PubMed: 2953813] [Abstract]
Cited for: NUCLEOTIDE SEQUENCE [MRNA].

[2] **"Structure and differential mechanisms of regulation of expression of a serine esterase gene in activated human T lymphocytes."**
Caputo A., Fahey D., Lloyd C., Vozab R., McCairns E., Rowe P.B.
J. Biol. Chem. 263:6363-6369(1988) [PubMed: 3258865] [Abstract]
Cited for: NUCLEOTIDE SEQUENCE [MRNA], VARIANTS ARG-55 AND ALA-94.

[3] **"Molecular cloning of an inducible serine esterase gene from human cytotoxic lymphocytes."**
Trapani J.A., Klein J.L., White P.C., Dupont B.
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[4] **"Genomic organization and chromosomal assignment for a serine protease gene (CSPB) expressed by human cytotoxic lymphocytes."**
Klein J.L., Shows T.B., Dupont B., Trapani J.A.
Genomics 5:110-117(1989) [PubMed: 2788607] [Abstract]
Cited for: NUCLEOTIDE SEQUENCE [GENOMIC DNA].

[5] **"Nucleotide sequence and genomic organization of a human T lymphocyte serine protease gene."**
Caputo A., Sauer D.E., Rowe P.B.
J. Immunol. 145:737-744(1990) [PubMed: 2365998] [Abstract]
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[6] **"Structural organization of the hCTLA-1 gene encoding human granzyme B."**
Haddad P., Clement M.-V., Bernard O., Larsen C.-J., Degos L., Sasportes M., Mathieu-Mahul D.
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[7] **"The DNA sequence and analysis of human chromosome 14."**
Heilig R., Eckenberg R., Petit J.-L., Fonknechten N., Da Silva C., Cattolico L., Levy M., Barbe V., De Berardinis V., Ureta-Vidal A., Pelletier E., Vico V., Anthouard V., Rowen L., Madan A., Qin S., Sun H., Du H. ⇔ Weissenbach J.
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[8] **"The status, quality, and expansion of the NIH full-length cDNA project: the Mammalian Gene Collection (MGC)."**
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Tissue: Pancreas.

[9] **"Isolation of a cDNA clone encoding a novel form of granzyme B from human NK cells**

and mapping to chromosome 14."
Dahl C.A., Bach F.H., Chan W., Huebner K., Russo G., Croce C.M., Herfurth T., Cairns J.S.
Hum. Genet. 84:465-470(1990) [PubMed: 2323780] [Abstract]
Cited for: NUCLEOTIDE SEQUENCE [MRNA] OF 1-23.

[10] **"Characterization of three serine esterases isolated from human IL-2 activated killer cells."**
Hameed A., Lowrey D.M., Lichtenheld M., Podack E.R.
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Cited for: PROTEIN SEQUENCE OF 21-40, CHARACTERIZATION.

[11] **"Characterization of granzymes A and B isolated from granules of cloned human cytotoxic T lymphocytes."**
Kraehenbuhl O., Rey C., Jenne D.E., Lanzavecchia A., Groscurth P., Carrel S., Tschopp J.
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[12] **"Human cytotoxic lymphocyte granzyme B. Its purification from granules and the characterization of substrate and inhibitor specificity."**
Poe M., Blake J.T., Boulton D.A., Gammon M., Sigal N.H., Wu J.K., Zweerink H.J.
J. Biol. Chem. 266:98-103(1991) [PubMed: 1985927] [Abstract]
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Zhang Z., Henzel W.J.
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[14] **"Crystal structure of the caspase activator human granzyme B, a proteinase highly specific for an Asp-P1 residue."**
Estebanez-Perpina E., Fuentes-Prior P., Belorgey D., Braun M., Kiefersauer R., Maskos K., Huber R., Rubin H., Bode W.
Biol. Chem. 381:1203-1214(2000) [PubMed: 11209755] [Abstract]
Cited for: X-RAY CRYSTALLOGRAPHY (3.1 ANGSTROMS) OF 21-247.

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J. Hum. Genet. 48:249-270(2003) [PubMed: 12721789] [Abstract]
Cited for: VARIANTS ARG-55 AND HIS-247.

\+ Additional computationally mapped references.

Cross-references

Sequence databases

EMBL	M17016 mRNA. Translation: AAA36627.1.

	J03189 mRNA. Translation: AAA36603.1. J04071 mRNA. Translation: AAA52118.1. J03072 Genomic DNA. Translation: AAB59528.1. M38193 Genomic DNA. Translation: AAA67124.1. M28879 Genomic DNA. Translation: AAA75490.1. AL136018 Genomic DNA. No translation available. BC030195 mRNA. Translation: AAH30195.1.
PIR	A61021.
RefSeq	NP_004122.1.
UniGene	Hs.1051

3D structure databases

PDB

Entry	Method	Resolution (Å)	Chain	Positions	PDBsum
1FQ3	X-ray	3.10	A/B	21-247	[»]
1IAU	X-ray	2.00	A	21-247	[»]

ModBase	Search...

Protein family/group databases

MEROPS	S01.010.

Proteomic databases

PRIDE	P10144.

Genome annotation databases

Ensembl	ENSG00000100453. Homo sapiens. [Contig view]
GeneID	3002.
KEGG	hsa:3002.

Organism-specific databases

GeneCards	GC14M024170.
H-InvDB	HIX0011578.
HGNC	HGNC:4709. GZMB.
HPA	CAB000376. HPA003418.
MIM	123910. gene.
PharmGKB	PA29087.
GenAtlas	Search...

Phylogenomic databases

HOVERGEN	P10144.

Enzyme and pathway databases

BRENDA	3.4.21.79; 247.
Reactome	REACT_578. Apoptosis.

Gene expression databases

ArrayExpress	P10144.
CleanEx	HS_GZMB.
GermOnline	ENSG00000100453. Homo sapiens.

Family and domain databases

InterPro	IPR001254. Peptidase_S1_S6. IPR001314. Peptidase_S1A. [Graphical view]
Pfam	PF00089. Trypsin. 1 hit. [Graphical view]
PRINTS	PR00722. CHYMOTRYPSIN.
SMART	SM00020. Tryp_SPc. 1 hit. [Graphical view]
PROSITE	PS50240. TRYPSIN_DOM. 1 hit. PS00134. TRYPSIN_HIS. 1 hit. PS00135. TRYPSIN_SER. 1 hit. [Graphical view]
ProtoNet	Search...

Other Resources

NextBio	11904.
SOURCE	Search...

Entry information

Entry name	GRAB_HUMAN
Accession	Primary (citable) accession number: **P10144** Secondary accession number(s): Q8N1D2
Entry history	Integrated into UniProtKB/Swiss-Prot: July 1, 1989 Last sequence update: July 1, 1989 Last modified: January 20, 2009 This is version 107 of the entry and version 1 of the sequence. [Complete history]
Entry status	Reviewed (UniProtKB/Swiss-Prot)
Annotation project	HPI (Human Proteome Initiative)

Relevant documents

Human chromosome 14 Human chromosome 14: entries, gene names and cross-references to MIM
Human entries with polymorphisms or disease mutations List of human entries with polymorphisms or disease mutations

Human polymorphisms and disease mutations
Index of human polymorphisms and disease mutations

MIM cross-references
Online Mendelian Inheritance in Man (MIM) cross-references in UniProtKB/Swiss-Prot

PDB cross-references
Index of Protein Data Bank (PDB) cross-references

Peptidase families
Classification of peptidase families and list of entries

SIMILARITY comments
Index of protein domains and families

Exhibit C

```
                    10        20        30        40        50        60
                     |         |         |         |         |         |
RATxxx0         MKLLLLLLLSFSLAPKTEAGEIIGGHEAKPHSRPYMAYLQIMDEYSGSKKCGGFLIREDFV
Humanx1         MQPILLLLAFLLLPRADAGEIIGGHEAKPHSRPYMAYLMIWDQKS-LKRCGGFLIQDDFV
                *: :*****:* * *:::********************** * *: *  *:******::***

                    70        80        90       100       110       120
                     |         |         |         |         |         |
RATxxx0         LTAAHCSGSKINVTLGAHNIKEQEKMQQIIPVVKIIPHPAYNSKTISNDIMLLKLKSKAK
Humanx1         LTAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAK
                ****** **.**************  **:*** : *******.*.:********:*: ***

                   130       140       150       160       170       180
                     |         |         |         |         |         |
RATxxx0         RSSAVKPLNLPRRNVKVKPGDVCYVAGWGKLGPMGKYSDTLQEVELTVQEDQKCESYLKN
Humanx1         RTRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRH
                *: **:**.** .:.:****:.* *****: .*:**:*.*****::*****:**** *::

                   190       200       210       220       230       240
                     |         |         |         |         |         |
RATxxx0         YFDKANEICAGDPKIKRASFRGDSGGPLVCKKVAAGIVSYGQNDGSTPRAFTKVSTFLSW
Humanx1         YYDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRACTKVSSFVHW
                *:*.: *:*.***:**::**:**********:*** ******:*:* .*** ****:*: *

RATxxx0         IKKTMKKS
Humanx1         IKKTMKRY
                ******:
Prim.cons.      IKKTMK22
```

Alignment data :
Alignment length : 248
Identity (*) : 170 is 68.55 %
Strongly similar (:) : 41 is 16.53 %
Weakly similar (.) : 12 is 4.84 %
Different : 25 is 10.08 %
Sequence 0001 : RATxxx0 (248 residues).
Sequence 0002 : Humanx1 (247 residues).

```
                    10         20         30         40         50         60
                     |          |          |          |          |          |
MOUSEx0         MKILLLLLLTLSLASRTKAGEIIGGHEVKPHSRPYMALLSIKDQQPEAICGGFLIREDFVL
Humanx1         MQPILLLLAFLLLPRADAGEIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
                *: :****:: * .*:.*********.********* * * **:.   ******::****

                    70         80         90        100        110        120
                     |          |          |          |          |          |
MOUSEx0         TAAHCEGSIINVTLGAHNIKEQEKTQQVIPMVKCIPHPDYNPKTFSNDIMLLKLKSKAKR
Humanx1         TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRPIPHPAYNPKNFSNDIMLLQLERKAKR
                ***** ** ************** ***.**: : **** ****.*********:*: ****

                   130        140        150        160        170        180
                     |          |          |          |          |          |
MOUSEx0         TRAVRPLNLPRRNVNVKPGDVCYVAGWGRMAPMGKYSNTLQEVELTVQKDRECESYFKNR
Humanx1         TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDLRHY
                ****:**.** .:.:****:.* *****: **:**:*:*****::***:**:*** :::

                   190        200        210        220        230        240
                     |          |          |          |          |          |
MOUSEx0         YNKTNQICVGDPKTKRASFRGDSGGPLVCKKVAAGIVSYGYKDGSPPRAFTKVSSFLSWI
Humanx1         YDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRACTKVSSFVHWI
                *:.* ::*****: *::**:*********:*** ****** ::* **** ******: **

MOUSEx0         KKTMKSS
Humanx1         KKTMKRY
                *****
Prim.cons.      KKTMK22
```

Alignment data :
Alignment length : 247
Identity (*) : 168 is 68.02 %
Strongly similar (:) : 38 is 15.38 %
Weakly similar (.) : 11 is 4.45 %
Different : 30 is 12.15 %
Sequence 0001 : MOUSEx0 (247 residues).
Sequence 0002 : Humanx1 (247 residues).